RESEARCH ARTICLE

Quantitative morphometrical analysis of a North African population of *Drosophila melanogaster*: sexual dimorphism, and comparison with European populations

M. CHAKIR[1], H. NEGOUA[1], B. MORETEAU[2] and J. R. DAVID[2,3]*

[1]*Laboratoire Aliment, Environnement et Santé, Faculté des Sciences et Techniques, Université Cadi Ayyad, BP 618, Marrakesh, Morocco*
[2]*Laboratoire Evolution, Génomes et Spéciation (LEGS), Centre National de la Recherche Scientifique, UPR 9034, Avenue de la Terrasse 91198, Gif-sur-Yvette Cedex, University Paris-Sud 11, 91405 Orsay Cedex, France*
[3]*Muséum National d'Histoire Naturelle (MNHN), Département Systématique et Evolution, UMR CNRS/MNHN 5202, GPS3, 45 rue Buffon 75005 Paris, France*

Abstract

Genetic variability of quantitative traits was investigated in a Moroccan population of *Drosophila melanogaster*, with an isofemale line design. Results were compared with data previously obtained from French populations. Although the environmental and thermal conditions are very different in France and Morocco, only two significant differences were observed: a shorter wing and a lighter abdomen pigmentation in Morocco. It is, therefore, concluded that Moroccan *D. melanogaster* are quite typical temperate flies, belonging to the Palaearctic region, and very different from the ancestral Afrotropical populations. Almost all traits were genetically variable, as shown by significant intraclass correlations among lines. Genetic correlations were highly significant among three size-related traits, while much lower between size and bristle numbers. Fluctuating asymmetry was greater for abdominal bristles than for sternopleural bristles. Sex dimorphism, analysed as a female/male ratio, was identical in French and Moroccan populations. Examination of the thorax length/thorax width ratio showed that the thorax is more elongated in females. Sexual dimorphism of wing length was significantly more correlated to thorax width than to thorax length. The results illustrate the value of measuring numerous quantitative traits on the same flies for characterizing the genetic architecture of a natural population. In several cases, and especially for genetic correlations, some interesting suggestions could be made, which should be confirmed, or invalidated, by more extensive investigations.

Introduction

Climatic adaptations, often revealed by latitudinal clines, are a fascinating research field in drosophilid flies. Attention has been mostly focussed on latitudinal size variations, asking the question: why is it better to be bigger in a colder environment? (Tantawy and Mallah 1961; Stalker 1980; David and Capy 1988; Capy *et al.* 1993; Atkinson 1994; Hoffmann and Parsons 1997; Azevedo *et al.* 1998). This is often called the temperature-size rule (Angilletta and Dunham 2003; Angilletta *et al.* 2004). There are, however, several difficulties in understanding such clinal trends. First, in some species, no clinal pattern has been found (Loeschke *et al.* 2000). Second, with respect to thermal phenotypic plasticity, the temperature-size rule is not strictly valid, especially for thorax size (David *et al.* 2006). Third, under the same thermal climatic conditions, the phenotypes may be quite different in distant geographic populations (Capy *et al.* 1993; Gilchrist and Partridge 1999). Fourth, numerous traits, besides body size, such as bristle number, ovariole number, pigmentation, ethanol tolerance, desiccation tolerance, exhibit

*For correspondence. E-mail: david@legs.cnrs-gif.fr.

latitudinal clines at least in *Drosophila melanogaster* (David and Capy 1988; Capy *et al*. 1993; Karan *et al*. 2000; David *et al*. 2004; Gibert *et al*. 2004). It is generally assumed that such traits, which are not directly related to size, may be a direct target of natural selection, even if the precise nature of environmental selective pressures is not always understood.

Facing such a range of quantitative variation among geographic populations, especially in *D. melanogaster*, there is a need for deeper and more accurate investigations on this paradigmatic species. Some pending questions are listed. (i) What is the stability of the characteristics of a local population when it is sampled over successive years? (ii) What is the best, most efficient sampling technique? (iii) Are the quantitative characteristics stable when the population is kept in the laboratory over several months or years? (iv) How many quantitative traits may be investigated as a routine in a given population? How are these traits correlated? What is the G matrix? (v) Can we detect significant and stable genetic differences between populations living in the same geographic area? In previous papers, we tried to answer the first three questions. For example, the stability of the French population was revealed in several investigations (Capy *et al*. 1993; David *et al*. 2006). The isofemale line design has been argued to be a very efficient, cost-effective technique for sampling a natural population (David *et al*. 2006). Laboratory drift is less documented but has been clearly observed for ovariole number (Bocquet *et al*. 1973). Numerous quantitative characters have been investigated in geographic populations of *D. melanogaster* and its sibling species *D. simulans* (David and Capy 1988; Capy *et al*. 1993; David *et al*. 2004; Gibert *et al*. 2004), but in many cases the different traits were not measured simultaneously on the same flies. The present paper is a first contribution trying to address questions (iv) and (v), i.e. the characterization of a Moroccan population which, although it belongs to the same biogeographic, Palaearctic region as France, breeds under different climatic conditions. While France is characterized by a cold winter and mild summer, Morocco is characterized by a mild winter and a very hot summer.

With an isofemale line design, we investigated a natural population from Marrakesh, Morocco. Several traits were simultaneously investigated: three size-related, metric traits, two meristic traits (bristle numbers on the thorax and abdomen), and abdominal tergite pigmentation. Three body-shape indices were also calculated. Most traits were measured on both sexes, and special attention was devoted to sex dimorphism.

We found that, in many respects, the Moroccan population was similar to the French ones, albeit with significant differences for wing/thorax ratio (wing loading) and abdomen pigmentation. Sex dimorphism was also stable across the Moroccan and French populations. Thorax width, analysed for the first time in a natural population, revealed an original difference among sexes: a more elongated thorax in female than in male.

Material and methods

Population investigated

Wild-living adults were collected using banana traps on the campus of the Cadi Ayyad University, Marrakesh, in March 2002. After anaesthesia, *D. melanogaster* females were sorted out from more frequent *D. simulans* females, using the shape of the black band on tergites five and six as a visual cue (Moreteau *et al*. 1995). These females were eventually isolated in culture vials to initiate isofemale lines. The species identity of each line was confirmed by examination of genitalia of male progeny, and 13 lines were finally used for the study.

Experimental flies

The adults of F_1, first laboratory generation, were used as parents of the experimental flies. From each line, 10 pairs were randomly taken, and set on ordinary *Drosophila*, sugar–cornmeal medium. After two days, the adults were transferred in vials containing a highly nutritive killed yeast medium, for about 6 h. Population density in each vial was not precisely controlled, but the rich, killed yeast food prevents larval crowding effects upon adult size (Karan *et al*. 1999). The egg laying protocol was repeated twice, on two successive days, and the experimental vials were kept at 25°C. When development was complete, the adults were transferred to ordinary food vials, at a lesser temperature (18–20°C), and measured after at least three days and not more than 12 days. All lines bred well, with each vial producing at least 50 adults, so that 10 females and 10 males were easily available in each line.

Traits investigated

Three kinds of traits (metric, meristic and pigmentation) were measured on 10 flies of each sex from each line. Size-related, metric traits were wing length (W) from the thoracic articulation to the tip, on a left side, lateral view; thorax length (T) also on lateral view, from the neck to the tip of scutellum; thorax width (t) measured from a ventral view as the distance between the bases of the two major, posterior sternopleural bristles. Measurements were done with an ocular micrometer in a binocular microscope, and values transformed into mm x100. While a huge literature exists on wing and thorax length in *D. melanogaster*, thorax width is rarely considered, and we are aware of only one such study (Cowley and Atchley 1988).

From the three size-related traits, we calculated three ratios. The W/T ratio characterizes the relative proportion of the wing with respect to the thorax. As shown in previous studies (Pétavy *et al*. 1997; David *et al*. 2006), this ratio is strongly negatively correlated with wing loading, and provides information on flight capacity. The thorax length/thorax width (T/t) ratio is clearly an elongation index, a higher value meaning the thorax is more elongated. The

third ratio (W/t) is calculated here for the first time, but its biological meaning is less obvious. Anyway, all three ratios provide information on the shape of the fly and can be designated as shape indices.

Two meristic, bristle number traits were measured: sternopleural (STP) bristle number on each side of the thorax. This is a classical, highly investigated trait in quantitative genetics studies (Mackay 2001, 2004). We also investigated the number of bristles on the seventh sternite (A7) of the abdomen, only in females.

Finally, abdomen pigmentation was analysed only in females, for segments five, six and seven. For each segment, the extension of the black pigment was estimated visually, according to phenotypic classes ranging from 0 (no pigment) to 10 (completely black), following David *et al*. (1990). For the sake of simplicity, we will consider here only the total pigmentation, i.e. the sum of the scores for the three segments.

Data analysis

Basic statistics (mean, variance, coefficient of variation, coefficient of correlation) were calculated with Statistica software (Statsoft 1999).

Due to the experimental design, within-line and between-line variability could be analysed by analysis of variance (ANOVA), permitting the calculation of an intraclass correlation coefficient which is akin to an isofemale line heritability (David *et al*. 2005). For the two meristic traits, values were available for each side of the body and we analysed the variations of the total bristle number and also the fluctuating asymmetry (FA). In the present paper, we also focussed on sexual dimorphism (SD) in homologous quantitative traits which can be measured on both sexes. Comparisons can be made by considering the mean values of the females and males of each line. There are two ways for describing SD: either a difference between female and male values (F–M) or a ratio of female to male values (F/M). We often consider both measures although, as discussed in previous papers (David *et al*. 2003; Huey *et al*. 2006) we prefer the ratio that has no dimensionality and allows comparisons between different characters.

For the two meristic bristle number traits, results were available for the left and right body side, so that the FA could be investigated in various ways (Palmer and Strobeck 1986; Pétavy *et al*. 2006).

Results

Basic statistical data

Results for mean values in females and males are given in table 1, which also provides the average within line CV, and two ways for describing the SD, that is the F/M ratio and the F–M difference. SD results will be discussed in the following section.

The three size related traits (wing length, W; thorax length, T; and thorax width, t) are much greater in females than in males, as usual in *D. melanogaster*. Also, the average wing length of the Moroccan population is significantly less than in the French populations, while thorax length is identical (David *et al*. 2003; Pétavy *et al*. 2004). This results in a lesser W/T ratio in Morocco (on an average 2.42) than in France (2.47) ($P < 0.01$). Thorax width (t) variability is investigated here for the first time in a natural population: hence, no comparative data exist. The within line variability of the three-size traits is similar with an average CV slightly less than two.

Sternopleural bristles have average values close to 18, with CVs close to 10: this is quite typical in *D. melanogaster* in temperate populations (Capy *et al*. 1993; Pétavy *et al*. 2006). To our knowledge, bristles on A7 segment were

Table 1. Basic statistics for the nine traits measured or calculated. Mean values (m) are given ± standard error (s.e.) calculated from 13-line values; the average, within-line coefficient of variation (CV) is also given. For the three size-traits, values are expressed in mm × 100; the three ratios are dimensionless; the two bristle numbers are the sum of left and right side. Pigmentation is the sum of the dark area on tergites five, six and seven. Sex dimorphism was calculated for each line, either as ratio female/male (F/M) or a difference female-male (F–M) and average values (± s.e.) are given.

| | Mean values | | | | Sex dimorphism | |
| | Females | | Males | | F/M ratio | F–M difference |
Traits	m	CV	m	CV	m	m
Wing length (W)	262.52 ± 1.17	1.58	226.92 ± 1.05	1.96	1.157 ± 0.004	35.600 ± 0.753
Thorax length (T)	108.71 ± 0.41	1.64	94.20 ± 0.30	2.19	1.154 ± 0.003	14.508 ± 0.307
Thorax width (t)	72.52 ± 0.27	1.86	65.65 ± 0.25	2.02	1.105 ± 0.004	6.869 ± 0.241
W/T	2.42 ± 0.01	1.30	2.41 ± 0.01	1.71	1.002 ± 0.003	0.006 ± 0.007
W/t	3.62 ± 0.01	1.74	3.46 ± 0.01	2.06	1.047 ± 0.002	0.163 ± 0.006
T/t	1.50 ± 0.00	1.54	1.44 ± 0.01	1.77	1.045 ± 0.003	0.064 ± 0.004
STP bristles	18.59 ± 0.26	9.01	17.78 ± 0.26	10.03	1.046 ± 0.010	0.808 ± 0.163
A7 bristles	11.10 ± 0.30	12.45	-	-	-	-	-
Pigmentation	10.35 ± 0.64	26.06	-	-	-	-	-

investigated for the first time in *D. melanogaster* by Araripe *et al*. (2008) in a French population, and the mean value (10.0) is close to that found in Morocco (11.1)

The pigmentation of the last three abdominal segments in females, with a mean of 10.35, is slightly lighter than in natural populations of France (Gibert *et al*. 1996; Pétavy *et al*. 2001).

Genetic variability between lines

All measured traits were submitted to a one-way ANOVA and in all cases, except for STP bristles, a highly significant line effect was observed, classically interpreted as a proof of a genetic heterogeneity among lines (David *et al*. 2005).

Table 2 summarizes these results by presenting the percentage of total variance explained by the between-line variability. On an average, the line effect accounts for about 30% of the total variability. Worth mentioning is the fact that six out of seven traits are more variable in females than in males. This cannot be considered as a significant difference, and a confirmation of this observation will be needed.

Table 2. Analysis of the between line genetic variability. Results of one-way ANOVA are simplified by giving only the percentage of total variance explained by the between-line effect. The intraclass correlation coefficients (ICC) (isofemale heritability) are also given.

	ANOVA% variance		ICC	
Traits	Females	Males	Females	Males
Wing length (W)	47.65***	40.42***	0.441	0.36
Thorax length (T)	37.27***	20.04**	0.324	0.126
Thorax width (t)	32.09***	29.28***	0.265	0.233
W/T	28.14***	20.09**	0.220	0.127
W/t	35.60***	28.10***	0.305	0.219
T/t	32.12***	36.78***	0.265	0.318
STP bristles	22.21	19.20	0.151	0.116
A7 bristles	34.33***	-	0.291	-
Pigmentation	41.42***	-	0.371	-

Level of significance, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Due to the fairly low number (13) of investigated lines, estimates of intraclass correlation (table 2) are not precise. However, in general, they are in agreement with the numerous previous estimates for *D. melanogaster*, and never reach 0.5 (Capy *et al*. 1994; David *et al*. 2003, 2006; Pétavy *et al*. 2004, 2006). Worth mentioning, however, are the very low values for STP numbers in both sexes and for thorax length in males. Whether these values are a mere consequence of the sampling process or reflect some peculiarity of the Moroccan populations would deserve further studies.

Correlations between different traits within each sex

According to the experimental protocol, we consider here two kinds of correlations, a within-line correlation, that mostly expresses an environmental component, and a between line correlation, which is closer to a genetic correlation

(David *et al*. 2005). Results are given in table 3. Only characters which were measured in both sexes are considered.

Table 3. Phenotypic (within-line) and genetic (between-line) correlations among the various traits investigated. The within-line correlation was calculated for each line, and average values with standard errors are given. The between-line correlation is based on 13 observations.

	within lines		between lines	
Traits	Females	Males	Females	Males
W–T	0.648 ± 0.064	0.659 ± 0.047	0.845	0.857
W–t	0.490 ± 0.064	0.459 ± 0.055	0.606	0.622
T–t	0.589 ± 0.078	0.634 ± 0.055	0.662	0.382
STP–W	-0.082 ± 0.117	0.087 ± 0.107	0.404	0.529
STP–T	-0.069 ± 0.102	0.048 ± 0.077	0.365	0.413
STP–t	0.100 ± 0.084	0.032 ± 0.076	0.144	0.516
Pigm–W	0.120 ± 0.115	-	-	0.06	-
A7–W	0.022 ± 0.112	-	-	-0.108	-
A7–Pigm	-0.176 ± 0.093	-	-	0.096	-

W, wing length; T, thorax length; STP, sternopleural bristles; A7, abdominal bristles on segment 7; Pigm, abdomen pigmentation in females (sum of scores of segments five, six and seven). Threshold of significance (at $P = 0.05$) for the *r* values depicted is 0.55.

Within lines, all size traits are positively correlated, with an average *r* value of 0.580 ± 0.034 ($n = 6$). Size is, however, not correlated to STP bristles (average *r* value: 0.019 ± 0.032; $n = 6$).

Between lines, we first notice a general trend of an increased value of the correlations. If we consider the three size related traits, the average between-line *r* value is 0.662 ± 0.072 ($n = 6$ in each case). For the correlations which involve STP, the average *r* value is 0.395 ± 0.057 ($n = 6$). This positive value is greater than zero.

Bilateral symmetry and FA

Numerous calculations can be done to describe bilateral symmetry and FA (Palmer and Strobeck 1986). For the sake of simplicity, we consider here only the left–right side (L.R) correlation, and an absolute scaled index (ASI) which is the absolute value of the L–R difference, divided by the mean bristle number, (L+R)/2 (Chakir *et al*. 2007). Results are given in table 4.

Within lines, the L.R correlation is significantly positive for STP bristles, although the average value (0.30) is fairly low. This shows that the differences among flies of the same line are significant, either due to genetic or to common garden effects, but that developmental instability (developmental noise) is predominant. When the means of the lines are considered, the environmental effects tend to disappear, hence much greater correlations, close to 0.70, are observed.

For the A7 bristles in females, an analogous but more pronounced effect is observed. First, the correlation at the within line level is close to zero: genetical differences among flies do not show up in the correlation. The genetic variation

Table 4. Analysis of bilateral symmetry in sternopleural (STP) and abdominal bristles on sternite 7 (A7). Results show values of the R.L correlation, and the value of the asymmetry standardised index (ASI).

		STP bristles		A7
		Females	Males	Females
Correlation R–L	Within lines	0.266 ± 0.108	0.278 ± 0.113	0.090 ± 0.087
	Between lines	0.771	0.680	0.827
ASI	Within lines	9.56 ± 0.77	11.12 ± 1.13	17.67 ± 1.12
	Between lines	3.38 ± 0.63	3.81 ± 0.71	5.50 ± 1.02

however appears at the between line level, and might be even greater ($r = 0.83$) than for STP bristles. This phenomenon is illustrated in figure 1. Such a difference between two kinds of bristles is surprising and should be confirmed by further studies.
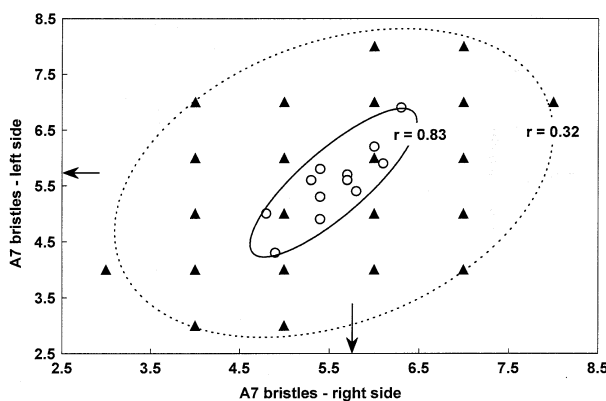

Figure 1. Correlation diagram between right and left side of abdominal bristles on sternite A7 in females. Ellipses of 80% confidence are shown, and correlation coefficients are given. Arrows point to the mean value on each side. Notice that the correlation of the total sample is 0.32, while the average within-line correlation is close to zero ($r = 0.090 \pm 0.087$; see table 4). Dark triangles, distribution of the total sample of 130 values. Open circles, distribution of the means of 13-isofemale lines.

The FA indices (ASI) were calculated either at the fly or at the line level and revealed a significant difference between STP and A7 bristles, the latter being more variable and less symmetrical than the former.

Sexual dimorphism

Several methods can be used to investigate sex dimorphism. A first approach is to consider the female/male correlation at the line level. Such a coefficient is akin to a genetic correlation and the values are given in table 5. For the three size traits, the positive correlations are similar (average value 0.66) and an illustration is provided in figure 2,A. Correlations are, however, very variable between shape indices (range 0.234–0.890) and the contrast between W/T and W/t is illustrated in figure 3. This difference in correlation is highly significant ($P < 0.01$). This result suggests that the

W/T ratio (related to wing loading) might be genetically independent in female and male. On the other hand, sex would determine, in a similar way in both sexes, the proportion between the wing and the thorax width.

STP bristles revealed a quite surprising observation. In the sample of lines investigated here, heritability seemed
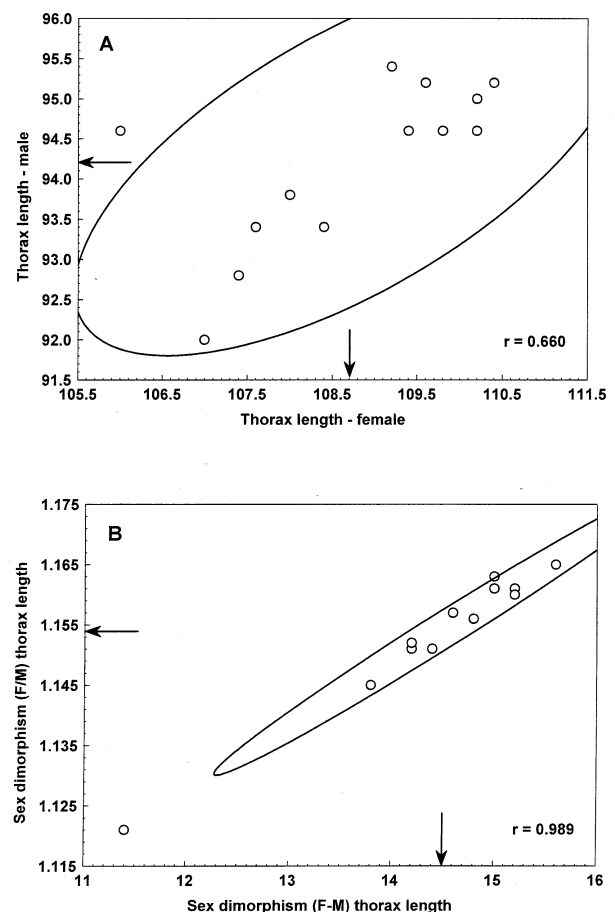

Figure 2. Analysis of thoracic sexual dimorphism (SD) among 13 isofemale lines. (A) Correlation between thorax length of female and male. (B) Correlation between two SD indices of thorax length. Arrows point to the mean value of each distribution. Ellipses of 80% confidence are shown to help visualize the distributions; correlation coefficients are also given. F–M, female minus male difference; F/M, female/male ratio.

Table 5. Analysis of sex dimorphism using correlations. Second column: correlation between female and male mean-line values. Third column: correlation between the two SD indices: F/M ratio and F–M difference.

Traits	Between means	Between indices
Wing length (W)	0.776**	0.980***
Thorax length (T)	0.660*	0.989***
Thorax width (t)	0.579*	0.996***
W/T	0.234^{ns}	0.999***
W/t	0.890***	0.995***
T/t	0.763**	0.998***
STP bristles	0.807***	0.997***

Level of significance: ns, nonsignificant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
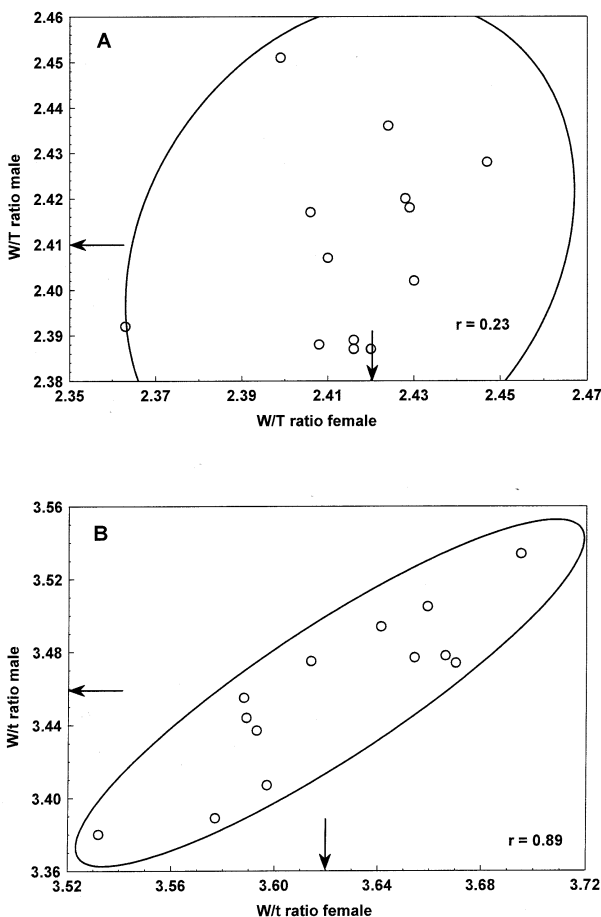

Figure 3. Correlation diagrams between shape indices in females and males. (A) Wing length/thorax length ratio (W/T). (B) Wing length/thorax width ratio (W/t). Ellipses of 80% confidence are shown; arrows point to the mean value of each distribution; correlation coefficients are given in each case. The difference between the two correlations is highly significant (*t* test after a *z* transformation, $P = 0.207$).

quite low, with an average value of 0.13 (table 2). In other words, genetic differences were small between lines. How-

ever, when these variations were correlated between sexes, a high coefficient was obtained ($r = 0.81$).

A second approach is to consider the best way to describe SD. Two possible indices were calculated, the F/M ratio and the F–M difference. These two indices are very highly correlated, with *r* values very close to one (table 5; figure 2,B). In other words, they provide the same information and, as in previous papers (e.g. David *et al*. 2003), we prefer the F/M ratio which is a nondimensional measure, not influenced by variations of the mean, and thus permitting a comparison of different traits.

Values of these indices are given in table 1. For wing and thorax length, the F/M ratios are the same, slightly greater than 1.15, and almost identical to the values found in a French population (David *et al*. 2003). The ratio for thorax width is, however, much less (1.10) which means that males are more similar to females for that trait.

This effect also appears when considering the ratio of thorax length to thorax width (T/t), i.e. the elongation index. This ratio (1.045) is greater than 1, meaning that male thorax is more rounded than the female. A similar value is found for the W/t ratio. The W/T ratio is, however, very close to one, and this is related to the fact that the two traits compared have identical dimorphisms.

For STP number, the dimorphism is significant but far less than expected from a geometrical point of view. Under this hypothesis, bristle number should be proportional to thoracic area, approaching the square of the ratio of linear dimensions, i.e. 1.33. In fact, the mean ratio found in Morocco is much less and is only 1.046, in agreement with values already found in *D. melanogaster* from France (Pétavy *et al*. 2006; David *et al*. 2006).

Genetic variability of SD among lines was analysed by calculating an intraclass correlation comparing the within-line and between-line variances. For this, we used the same artifice as in David *et al*. (2003) and Araripe *et al*. (2008). Within each line, the value of each female was divided by the mean of their brothers, thus providing a within line variance. Calculated intraclass correlations are given in table 6: with the exception of W/t ratio, they are all significantly greater than zero, but the overall mean value (0.17) seems, on average, lower than for the traits themselves (table 2).

We got surprising results for STP bristles. Within each sex, variability among lines was reduced, resulting in a low isofemale heritability (ICC = 0.13) (table 2). When, however, female and male data were compared, a high correlation was found ($r = 0.81$; table 5) suggesting that variations among lines, although small, had a real genetic basis, expressed in a similar way in both sexes. This results in a relatively high value of SD heritability, on average 0.34.

Finally, we asked the question: are the SD indices of different traits correlated? There are four kinds of possible correlations here, when either two identical SD indices are correlated (e.g. two ratios) or when the ratio of a trait (e.g. the wing) is correlated to the difference of the other trait

Table 6. Analysis of SD genetic variability considering either the F/M ratio or the F–M difference. ANOVA shows the percentage of variance explained by the between line variability. ICC is intraclass coefficient of correlation.

Trait	% Variance explained		ICC	
	F/M ratio	F–M diff.	F/M ratio	F–M diff.
Wing length (W)	29.88***	27.39***	0.240	0.211
Thorax length (T)	26.08***	25.29***	0.196	0.187
Thorax width (t)	28.59***	26.76***	0.225	0.204
W/T	38.52***	38.61***	0.338	0.339
W/t	11.04ns	10.57ns	0.021	0.015
T/t	24.09***	22.66***	0.173	0.157
STP bristles	35.83***	42.55***	0.308	0.383

Level of significance, ns: nonsignificant; ***$P < 0.001$.

(e.g. the thorax). We found (table 7) that the results were almost identical, regardless of whether ratios or differences were considered. For size traits, the lowest correlation was between SDs of wing and thorax length (average 0.485) and the highest between wing length and thorax width (average 0.863). This difference is illustrated in figure 4 and, despite the low number of lines, is marginally significant (*t* test after *z* transformation, $P = 0.049$). This observation suggests again that sex more directly affects the relationship between wing size and thorax width than that between wing and thorax length.

SD for STP bristles was not correlated to wing SD (average $r = 0.06$) but seemed to be correlated positively to thoracic SDs, either length or width (average $r = 0.31$). Such a low value is, however, not significantly greater than zero; hence, further investigations should be undertaken to confirm this conjecture.

Discussion

A morphometrical, genetic analysis was performed on 13 isofemale lines from a Moroccan population of *D. melanogaster*. This fairly small number is due to the relative rarity of *D. melanogaster* in Morocco, its sibling species *D. simulans* being much more abundant. The results are interesting for several reasons, including an isofemale line design, an analysis of recently collected flies in the second, laboratory generation, and the measure of a diversity of traits on both sexes. Several points will be discussed here.

Latitudinal variations

As indicated in the Introduction, latitudinal variations of body size are a powerful argument for assuming they are the consequence of a climatic adaptation, temperature playing a major role in this process. Significant regressions are more easily found when a broad range of latitudes are compared (Capy *et al.* 1993; Chakir *et al.* 1995; Gibert *et al.* 2004). However, significant differences should also be found over shorter distances, if environmental temperature plays a significant role.

In this respect, the population from Marrakesh may be compared to a French (Bordeaux) population studied by David *et al.* (2003). In Bordeaux, the winter temperature is much colder, i.e. 8.2°C for the three colder months, while in Marrakesh, the corresponding mean value is 13°C. In *D. melanogaster*, a complete development is possible at 12°C, so that winter generations, as well as summer generations are possible in Morocco (Chakir *et al.* 2007). In France, on the other hand, development is not possible during winter and over-wintering is likely due to the survival of adults in sheltered places (David *et al.* 1983, 2004; Pétavy *et al.* 2001).

In a previous study (David and Bocquet 1973), several Moroccan populations were compared with French and Afrotropical ones, for two metrical traits: body weight and ovariole number. Significant differences were observed between the three sets of populations, but the Moroccan flies were more similar to that of French than the Afrotropical ones.
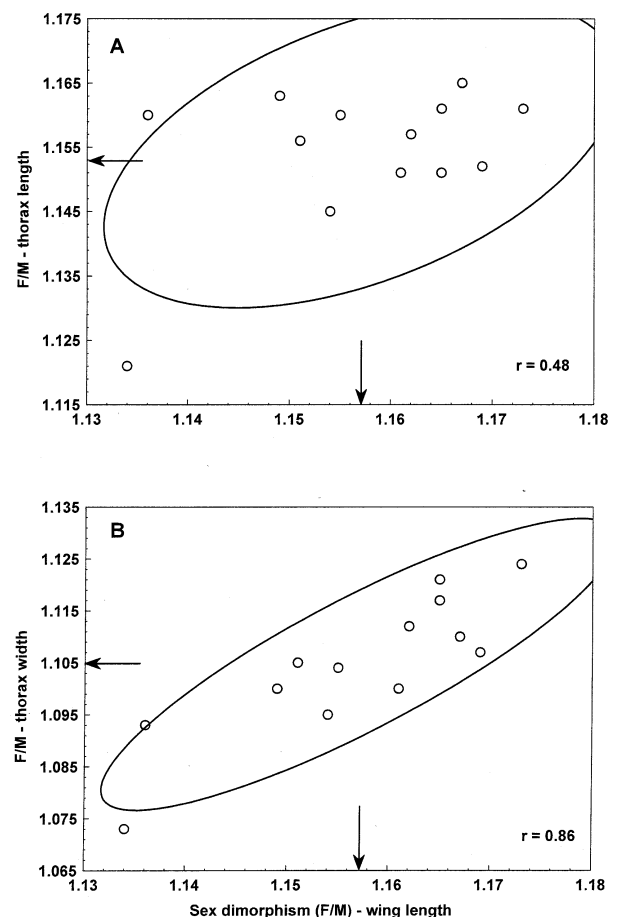

Figure 4. Correlation diagrams between sex dimorphism (SD) indices of different traits. (A) Relationship between SDs of wing length and thorax length. (B) Relationship between SDs of wing length and thorax width. Ellipses of 80% confidence are shown; arrows point to the mean values; coefficients of correlation are given.

Table 7. Correlation between SD indices of different characters using either the F/M ratio or the F–M difference.

Traits	Ratio × ratio	Diff × diff	Ratio × diff	Diff × ratio	Mean ± s.e.
W-T	0.48	0.50	0.47	0.485	0.49 ± 0.006
W-t	0.87	0.86	0.85	0.863	0.86 ± 0.005
T-t	0.70	0.73	0.72	0.713	0.71 ± 0.008
STP-W	0.06	0.06	0.06	0.060	0.06 ± 0.000
STP-T	0.34	0.35	0.35	0.345	0.35 ± 0.003
STP-t	0.28	0.26	0.27	0.270	0.27 ± 0.004

Threshold of significance for the *r* values depicted: 0.55 (at $P = 0.05$); 0.68 (at $P = 0.01$); 0.80 (at $P = 0.001$).

In the present work, we found some other significant differences between French and Moroccan flies. For size traits, a clear difference has been found for the wing, but not for the thorax. As a consequence, the W/T ratio is significantly less in Morocco, which implies a higher wing loading. This agrees with the hypothesis that, during the latitudinal expansion of the species, selection occurred mostly on wing size, in order to favour the capacity to fly in a cold environment, using a lesser wing-beat frequency (Stalker 1980; Pétavy *et al.* 1997). We also found that body pigmentation was lighter in Morocco than in France, in agreement with the thermal budget hypothesis (Gibert *et al.* 1996). This also agrees with the fact that, in the sibling *D. simulans*, a significant difference in pigmentation was found between winter and summer generations in Morocco (Chakir *et al.* 2007). Both W/T and body pigmentation may be considered as fast evolving traits, adapting rapidly to local temperature change. No significant difference was, however, found for thorax length and sternopleural bristles. In conclusion, the Moroccan populations must be considered as temperate populations, belonging to the Palaearctic region, as also demonstrated by a cold tolerance of spermatogenesis (David *et al.* 2005) and also by microsatellite analysis (Dieringer *et al.* 2005).

Body shape and indices

In all analyses of shape, a major problem is to eliminate, at least in part, the influence of size and of allometric relationships. A traditional approach in *Drosophila* has been to calculate a diversity of taxonomic indices (Sturtevant 1942; Burla 1954). In this respect, the W/T ratio is clearly a shape index. The reduction of variability in ratios is evidenced by a decrease of the within-line CVs (table 1). Besides wing and thorax length, we also measured thorax width and this permitted the calculation of two other ratios, among which the thorax length to width ratio is obviously an elongation index of the thorax. The values in *D. melanogaster* (1.50 in females, 1.44 in males) are calculated here for the first time. A similar study on a distantly related drosophilid (*Zaprionus indianus*) (Yassin *et al.* 2007) yielded a much greater value, close to 1.75, suggesting that thorax shape variability might be an interesting trait to study in drosophilid evolution.

An interesting observation on all these ratios is that they are genetically variable, with fairly high isofemale heritabilities (table 2). The capacity of these ratios to evolve across species or to respond to environmental variation will be interesting to investigate from a comparative point of view.

Correlations between traits in the same sex

At the within-line level, 13 independent correlation coefficients were available providing a fairly precise average value. The main conclusion is that similar traits (e.g. body size-related) are positively and highly correlated, while different types of traits (e.g., size and bristles number) are not.

At the between-line level, only a single *r* value is available, hence a lack of precision. There is a general tendency for these genetic correlations to be higher than the within-line correlations. This is expected, considering that the influence of random fluctuations tends to disappear when line mean values are considered. All correlations between size traits are significant, while those involving other traits are not. An interesting observation, however, is the fairly high correlation (average $r = 0.395 ± 0.057$, $n = 6$) observed between size traits and STP bristles, which should be investigated in other populations to assess its generality.

Fluctuating asymmetry (FA)

There are only a few publications in which the bilateral symmetry of meristic traits has been investigated in *Drosophila* (Shakarad *et al.* 2001; Pétavy *et al.* 2006; Chakir *et al.* 2007). The left–right side (L.R) correlation (table 4) showed a higher value between lines as compared to within lines, and this may be considered an expected consequence of genetic differences in bristle number among lines. We also found an unexpected difference between STP bristles and the bristles on abdominal sternite A7. For these abdominal bristles, the within-line correlation L.R was zero, contrasting with a high correlation among lines. The FA index (ASI) was also much greater for A7 than for STP bristles, again suggesting a stronger instability for the abdominal bristles. Interestingly, when ASIs of the two kinds of bristles were correlated, a nil value was observed, indicating that the magnitude of FA in

the thorax does not influence that at the tip of the abdomen, and vice versa.

Sexual dimorphism

Sexual dimorphism is genetically variable for almost all traits, as shown by significant intraclass correlation coefficients (table 5). This conclusion is confirmed by positive female-male correlations for the same trait. Interestingly, isofemale line heritabilities for SD are generally lower than for the traits themselves, as expected (David *et al*. 2003; Pétavy *et al*. 2004, 2006), but not by too much. There is, therefore, a large genetic potential for SD indices to change between populations. Our results, however, revealed a remarkable identity of body size SD (wing and thorax length) between French (David *et al*. 2003) and Moroccan flies. In a recent, similar investigation on a cosmopolitan drosophilid *Zaprionus indianus* (David *et al*. 2006), SD for body size was found to be very low but remarkably constant among geographic populations all over the world. By contrast, old laboratory strains were found to be more variable with respect to SD. These observations were interpreted as an indication of a strong balancing selection in nature, but a consequence of drift in laboratory conditions. The same phenomenon might exist in *D. melanogaster*, since we have some evidence that old laboratory cultures are much more variable than recently collected populations (J. R. David, unpublished data).

A major evolutionary problem in the future will be to understand how the sex-determining genetic cascade interferes with various quantitative traits. A possible approach is to consider genetic correlations of SD indices for different traits. High positive significant values have been found for the SD of size traits, and this is expected under the assumption that all these traits share a common genetic basis. Knowing whether wing dimorphism is more correlated to that of thorax width than thorax length would need more extensive investigation. Another suggestion from our data would be that STP dimorphism is not correlated to wing SD, but slightly positively to SD of thorax dimensions. This would make sense from an anatomical point of view.

Finally, the analysis of thorax width (t) has revealed a significant, unexpected dimorphism. When compared to the thorax length (T/t ratio), SD is significantly greater than 1: the thorax is more elongated in the female, and this might influence the flight capacity between sexes.

References

Angilletta M. J. and Dunham A. E. 2003 The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. *Am. Nat.* **162**, 332–342.

Angilletta M. J., Steury T. S. and Sears M. W. 2004 Temperature growth rate and body size in ectotherms: fitting pieces of a life history puzzle. *Integr. Comp. Biol.* **44**, 498–509.

Araripe L. O., Yassin A., Klaczko L. B., Moreteau B. and David J. R. 2008 Divergent abdominal bristle patterns in two distantly related drosophilids: antero–posterior variations and sexual dimorphism in a modular trait. *Genetica* **134**, 211–222.

Atkinson D. 1994 Temperature and organism size – a biological law for ectotherms? *Adv. Ecol. Res.* **25**, 1–58.

Azevedo R. B. R., James A. C., McCabe J. and Partridge L. 1998 Latitudinal variation of wing:thorax size ratio and wing-aspect ratio in *Drosophila melanogaster. Evolution* **52**, 1253–1362.

Bocquet C., David J. R. and De Scheemacker-Louis M. 1973 Variabilite du nombre d'ovarioles des souches sauvages de *Drosophila melanogaster* conservees en laboratoire sans selection volontaire. *Arch. Zool. Exp. Gen.* **114**, 475–489.

Burla H. 1954 Zur Kenntnis der Drosophiliden der elfenbeinkuste (Franzosisch West-Africa). *Rev. Suisse Zool.* **61**, 1–218.

Capy P., Pla E. and David J. R. 1993 Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans.* I. Geographic variations. *Genet. Sel. Evol.* **25**, 517–536.

Capy P., Pla E. and David J. R. 1994 Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans.* II. Within population variability. *Genet. Sel. Evol.* **26**, 15–28.

Chakir M., David J. R. and Capy P. 1995 Genetic basis of some morphological differences between temperate and equatorial populations of *Drosophila melanogaster. Experientia* **51**, 744–748.

Chakir M., Moreteau B., Capy P. and David J. R. 2007 Phenotypic variability of wild living and laboratory grown *Drosophila*: consequences of nutritional and thermal heterogeneity in growth conditions. *J. Therm. Biol.* **32**, 1–11

Cowley D. E. and Atchley W. R. 1988 Quantitative genetics of *Drosophila melanogaster.* II. Heritabilities and genetic correlations between sexes for head and thorax traits. *Genetics* **119**, 421–433.

David J. R. and Bocquet C. 1973 Sur certains caracteres quantitatifs des souches de *Drosophila melanogaster* provenant du sud du Maroc. *C. R. Acad. Sci. Paris* **277**, 877–880.

David J. R. and Capy P. 1988 Genetic variation of *Drosophila melanogaster* natural populations. *Trends Genet.* **4**, 106–111.

David J. R., Allemand R., Van Herrewege J. and Cohet Y. 1983 Ecophysiology: abiotic factors. In *Genetics and biology of Drosophila* (ed. M. Ashburner, H. L. Carson and J. N. Thompson Jr), pp. 105–170. Academic Press, New York.

David J. R., Capy P. and Gauthier J. P. 1990 Abdominal pigmentation and growth temperature in *Drosophila melanogaster*: similarities and differences in the norms of reaction of successive segments. *J. Evol. Biol.* **3**, 429–445.

David J. R., Gibert P., Mignon-Grasteau S., Legout H., Pétavy G., Beaumont C. and Moreteau B. 2003 Genetic variability of sexual sex dimorphism in a natural population of *Drosophila melanogaster*: an isofemale line approach. *J. Genet.* **82**, 101–110.

David J. R., Allemand R., Capy P., Chakir M., Gibert P., Pétavy G. and Moreteau B. 2004 Comparative life histories and ecophysiology of *Drosophila melanogaster* and *D. simulans. Genetica* **120**, 151–163.

David J. R., Gibert P., Legout H., Capy P. and Moreteau B. 2005 Isofemale lines in *Drosophila*: an empirical approach to quantitative traits analysis in natural populations. *Heredity* **94**, 3–12.

David J. R., Legout H. and Moreteau B. 2006 Phenotypic plasticity of body size in a temperate population of *Drosophila melanogaster*: when the temperature-size rule does not apply.

J. Genet. **85**, 9–23.

David J. R., Araripe L., Bitner-Mathe B. C., Capy P., Goni B., Klaczko L. B. *et al*. 2006 Sexual dimorphism of body size and sternopleural bristle number: a comparison of geographic populations in an invasive cosmopolitan drosophilid. *Genetica* **128**, 109–122.

Dieringer D., Nolte V. and Schlötterer C. 2005 Population structure in African *Drosophila melanogaster* revealed by microsatellite analysis. *Mol. Ecol.* **14**, 563–573.

Gibert P., Moreteau B., Moreteau J. C. and David J. R. 1996 Growth temperature and adult pigmentation in two *Drosophila* sibling species: an adaptive convergence of reaction norms in sympatric populations. *Evolution* **50**, 2346–2353.

Gibert P., Capy P., Imasheva A., Moreteau B., Morin J. P., Pétavy G. and David J. R. 2004 Comparative analysis of morphological traits among *Drosophila melanogaster* and *D. simulans*: genetic variability, clines and phenotypic plasticity. *Genetica* **120**, 165–179.

Gilchrist A. S. and Partridge L. 1999 A comparison of the genetic basis of wing size divergence in three parallel body size clines of *Drosophila melanogaster*. *Genetics* **153**, 1775–1787.

Hoffmann A. A. and Parsons P. A. 1997 *Extreme environmental change and evolution.* Cambridge University Press, Cambridge.

Huey R. B., Moreteau B., Moreteau J. C., Gibert P., Gilchrist G. W., Ives A. R. *et al*. 2006 Sexual size dimorphism in a *Drosophila* clade, the *D. obscura group. Zoology* **109**, 318–330.

Karan D., Morin J. P., Gravot E., Moreteau B. and David J. R. 1999 Temporal stability of body size reaction norms in a natural population of *Drosophila melanogaster*. *Genet. Sel. Evol.* **31**, 491–508.

Karan D., Dubey S., Moreteau B., Parkash R. and David J. R. 2000 Geographical clines for quantitative traits in natural populations of a tropical drosophilid: *Zaprionus indianus. Genetica* **108**, 91–100.

Loeschke V., Bundgaard J. and Barker J. S. F. 2000 Variation in body size and life history traits in *Drosophila aldrichi* and *D. buzzatii* from a latitudinal cline in eastern Australia. *Heredity* **85**, 423–433.

Mackay T. F. C. 2001 The genetic architecture of quantitative traits. *Annu. Rev. Genet.* **35**, 303–339.

Mackay T. F. C. 2004 The genetic architecture of quantitative traits: lessons from *Drosophila. Curr. Opin. Genet. Dev.* **14**, 253–257.

Moreteau B., Pétavy G., Gibert P., Morin J. P., Munoz A. and David J. R. 1995 New discriminating traits between females of two sibling species: *Drosophila melanogaster* and *D. simulans* (Diptera, Drosophilidae). *Ann. Soc. Entomol. Fr. (N.S.)* **31**, 249–257.

Palmer A. and Strobeck C. 1986 Fluctuating asymmetry: measurement, analysis, patterns. *Annu. Rev. Ecol. Syst.* **17**, 391–421.

Pétavy G., Morin J. P., Moreteau B. and David J. R. 1997 Growth temperature and phenotypic plasticity in two *Drosophila* sibling species: probable adaptive changes in flight capacities. *J. Evol. Biol.* **10**, 875–887.

Pétavy G., David J. R., Gibert P. and Moreteau B. 2001 Viability and rate of development at different temperatures in *Drosophila*: a comparision of constant and alternating thermal regimes. *J. Therm. Biol.* **26**, 29–39.

Pétavy G., David J. R., Debat V., Gibert P. and Moreteau B. 2004 Specific effects of cycling stressful temperatures upon phenotypic and genetic variability of size traits in *Drosophila melanogaster. Evol. Ecol. Res.* **6**, 873–890.

Pétavy G., David J. R., Debat V., Pertoldi C. and Moreteau B. 2006 Phenotypic and genetic variability of sternopleural bristle number in *Drosophila melanogaster* under daily thermal stress: developmental instability and anti-asymmetry. *Evol. Ecol. Res.* **8**, 1–19.

Shakarad M., Prasad N. G., Rajamani M. and Joshi A. 2001 Evolution for faster development does not lead to greater fluctuating asymmetry of sternopleural bristle number in *Drosophila. J. Genet.* **80**, 1–7.

Stalker H. D.1980 Chromosome studies in wild populations of *Drosophila melanogaster*. II. Relashionship of inversion frequencies to latitude, season, wing-loading and flight activity. *Genetics* **95**, 211–223.

Statsoft 1999 *Statistica*, Version 5.5. Statsoft, Tulsa.

Sturtevant A. H. 1942 The classification of the genus *Drosophila*, with descriptions of nine new species. *Univ. Texas Publ.* **4213**, 5–51.

Tantawy A. O. and Mallah G. S. 1961 Studies on natural populations of *Drosophila*. I. Heat resistance and geographical variation in *D. melanogaster* and *D. simulans. Evolution* **15**, 1–14.

Yassin A., Abou-Youssef A., Bitner-Mathe B. C., Capy P. and David J. R. 2007 Developmental stress in wild-living Drosophilids inferred from biometry: metric and meristic traits react differently to heterogeneous environmental conditions. *Ecol. Entomol.* **32**, 1–19.